TRANSFER OF ELECTRONIC EXCITATION ENERGY FROM β-CAROTENE TO CHLOROPHYLL-a IN SOLUTION

By
J. SZABAD

Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Szeged

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From the sensitization of chlorophyll fluorescence by β-carotene the critical distance of energy transfer R_0 was found to be dependent on the wavelength of exciting light in the region 400—440 nm, ranging from 18 Å to 22 Å in the Soret band of chlorophyll-a. In the region 450—500 nm (where the absorption is mainly due to β-carotene) $R_0 = 25$ Å. The value $R_0 = 8{,}5$ Å was obtained from the absorption and fluorescence spectra. The superposition of a "fast" transfer of energy involving the second excited singlet state of chlorophyll-a is offered as explanation.

Introduction

It is known that the electronic excitation energy of carotenoids is transferred to chlorophyll-a (Chl-a) and is useful in inducing photosynthesis [1—3]. In mixed solutions containing detergent, in monolayers containing carotene and Chl-a and in thin films, certain carotenoids are active in exciting Chl-a fluorescence [4—6]. In mixed solutions of β-carotene and Chl-a in castor oil the energy transfer was observable only at low temperature [3]. SINGHAL and co-workers [7] supposed that β-carotene in benzene and in 3-methoxy-1-butanol was ineffective in exciting fluorescence of Chl-a, but they did not exclude the possibility of energy transfer from β-carotene to Chl-a in other solvents.

DUYSENS [1] and RABINOWITCH [8] assume that the transfer of energy from carotenoids to chlorophylls takes place according to an inductive resonance mechanism obeying Förster's formulas [9]. LOSEV and GURINOVICH [3] and SINESHCHEKOV *et al.* [6] suppose that the mechanism of carotenoid → Chl energy transfer is not inductive resonance but something different. According to LOSEV and GURINOVICH [3] an effective carotenoid → Chl energy transfer occurs if the system contains some type of "solid support surface", as in detergent solutions and in monolayers. There are cases in which the carotenoid → Chl energy transfer can not be explained by Förster's theory, since in a certain wavelength region of excitation the efficiency of transfer depends on the wavelength [10]. If Förster's theory is formally applied to the transfer at different exciting wavelengths, this dependence leads to different transfer frequencies and critical distances not predicted by this theory. A similar effect was observed by WEBER [11] and BAUER *et al.* [12]. In this paper β-carotene → Chl-a energy transfer will be discussed in terms of the critical distances of transfer from sensitization of Chl-a fluorescence, and of absorption and fluorescence spectra.

Material and Methods

Chl-a was prepared from fresh spinach leaves according to JACOBS *et al.* [13]. The Chl-a solutions in diethyl ether were considered pure if the intensity ratios of the blue and red absorption maxima and those of red absorption maxima to the absorption at 505 nm were 1,32 and 45—50 respectively. β-carotene was a Merck product. The absorption spectrum of β-carotene showed that the preparation consisted only of the trans compound [14]. The mixed cyclohexane solutions contained 10^{-5} M Chl-a and $4\cdot10^{-6}$, $6\cdot10^{-6}$, $8\cdot10^{-6}$, 10^{-5}, $2\cdot10^{-5}$, $4\cdot10^{-5}$, $6\cdot10^{-5}$, $8\cdot10^{-5}$ and 10^{-4} M β-carotene. All measurements were carried out at room temperature.

The critical distance (the average distance of donor and acceptor when the probability of transfer is equal to that of emission) is given by Eq. (1) [9]

$$R_0^6 = \frac{9(\ln 10)k^2c^4\Phi_d}{128\,\pi^5 n^4 N'}\int_0^\infty f(\nu)\varepsilon(\nu)\frac{d\nu}{\nu^4}. \tag{1}$$

Here $k^2=2/3$ (for random distribution of the molecules), c is the velocity of light, Φ_d the fluorescence yield of the donor, n is the refractive index of the medium, N' is the number of molecules per mmole, $f(\nu)$ the normalized fluorescence spectrum of the donor, $\varepsilon(\nu)$ is the molar decadic extinction coefficient of the acceptor and ν the frequency. The fluorescence spectrum of β-carotene was taken from the literature [15]. Φ_d was taken as 10^{-4} [1]. According to CHERRY *et al.* [15], the yield of fluorescence of β-carotene is 1/500 of that of anthracene. The fluorescence spectra were normalized under the condition $\int_0^\infty f(\nu)d\nu=1$.

The critical distance can also be determined from quenching curves given by the following equation [9]:

$$\frac{\Phi}{\Phi_0} = 1-\sqrt{\pi}\,\frac{c_M}{c_0}\cdot e^{\left(\frac{c_M}{c_0}\right)^2}\left[1-\varphi\left(\frac{c_M}{c_0}\right)\right], \tag{2}$$

where c_M is the concentration in mole/l, c_o the critical concentration (at which the probability of energy transfer is equal to that of emission) and $\varphi(x)$ is the Gaussian error integral. Φ_0 and Φ represent the fluorescence yield when the fluorescence of the donor is not quenched and quenched, respectively. The relation between R_0 and c_0 is

$$R_0^3 = \frac{3}{2\sqrt{\pi^3 N' c_0}}. \tag{3}$$

As the yield of fluorescence of β-carotene is very low, Φ/Φ_0 is not measurable for β-carotene. However, the sensitization of the fluorescence of Chl-a by β-carotene and the quenching of the fluorescence of β-carotene by Chl-a are different manifestations of the same process, and therefore from Chl-a fluorescence sensitization "quenching curves" for β-carotene fluorescence can be determined. The sensitization of Chl-a fluorescence in mixed solutions of carotene and Chl is usually characterized by the ratio F'_m/F'_c, where F'_m is the measured and F'_c the calculated fluorescence intensity of Chl in the mixed solution [7], [10]. F'_c/F'_m may represent the "quenching of fluorescence" of β-carotene.

Results

Using Eq. (1) R_0 was calculated for four pigment pairs (Table 1). The spectra shown in Fig. 1 were used for the calculation. The red absorption band of Chl-a was taken into account, the higher frequency side of which is shown by a dotted

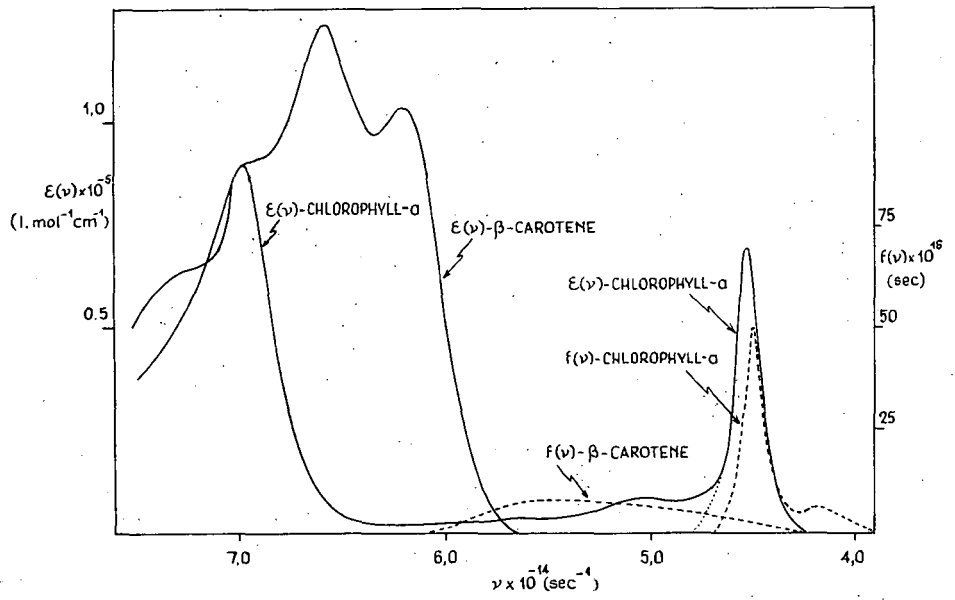

Fig. 1. Absorption and fluorescence spectra of β-carotene and chlorophyll-a in cyclohexane

line. In Fig. 2 the "quenching curves" of β-carotene fluorescence are shown for four exciting wavelengths (actually the reciprocals of the intensities of the relative fluorescence of Chl-a). From these curves the critical concentrations, and with Eq. (3) the critical distances were calculated. For the β-carotene Chl-a system in

Table I

R_0 values in Å from the spectra for four pigment pairs. R_0 = 25 Å calculated from "quenching curves" in the wavelength region 440—500 nm

Donor \ Acceptor	Chlorophyll-a	β-carotone
Chlorophyll-a	43,6	0
β-carotene	8,5 (25,0)	6,5

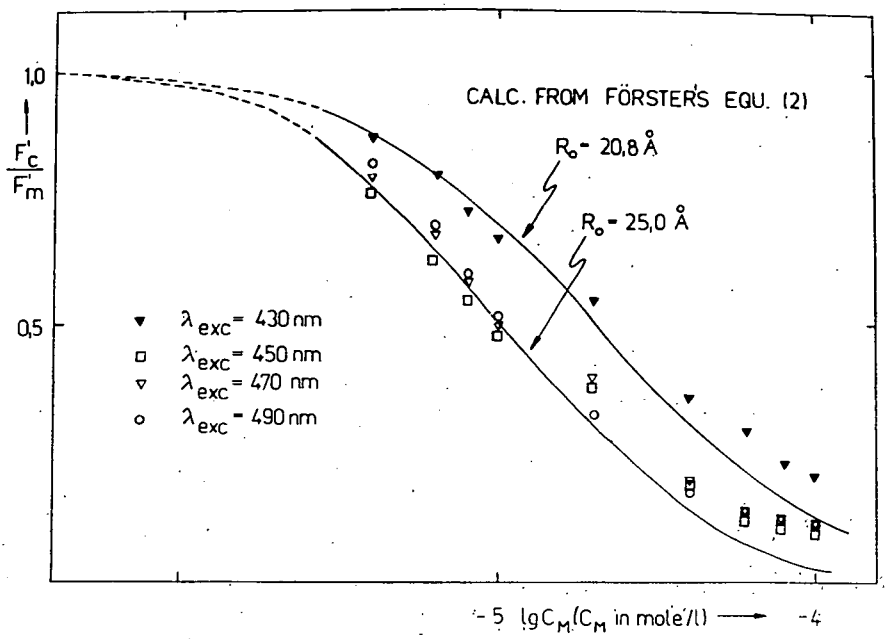

Fig. 2. Reciprocal of relative fluorescence intensities of chlorophyll-a β-carotene mixtures as function of the concentration of β-carotene

the region 450—500 nm, R_0 was obtained as 25 Å independently of the wavelength of excitation. At exciting wavelengths in the region where the absorption of Chl-a is significant, c_0 and, consequently R_0 depend on the wavelength of the excitation, ranging from 18—22 Å.

Discussion

Table I shows that, in principle, according to Förster theory the transfer from β-carotene to Chl-a in cyclohexane solution is possible (with R_0=8.5 Å); its efficiency under the present conditions, however, is negligible. β-carotene is not able to quench the fluorescence of Chl-a (R_0=0 Å), *i.e.* the β-carotene → Chl-a transfer is irreversible. There is practically no transfer of energy involving β-carotene molecules (R_0=6.5 Å).The "fluorescence quenching curves", however, show that in solution β-carotene transfers its electronic excitation energy to Chl-a (R_0=25 Å).

R_0 calculated from absorption and fluorescence spectra is much less than that obtained from the "quenching". This difference can be explained in two ways:

1. The fluorescence yield of β-carotene introduced into Eq. (1) should be much higher than 10^{-4}. If this were the cause of the difference, an absolute yield of fluorescence of β-carotene of about $1{,}6 \cdot 10^{-3}$ should be expected; this may be the case because the fluorescence of β-carotene, also under these conditions, is not easily measurable.

2. There exists some interaction between Chl-a and β-carotene molecules promoting the carotenoid → Chl energy transfer, *e.g.* the presence of mixed dimers or

higher aggregates. However there is no sign of these aggregates in the absorption and fluorescence spectra of mixed solutions.*

The R_0 values calculated from the "quenching curves" show the following characteristics. In the wavelength region where mainly β-carotene absorbs, R_0 does not depend on the wavelength of excitation. In the region, where Chl-a also absorbs (400—440 nm), however, R_0 depends on the wavelength. R_0 in this region is a formal quantity, since this dependence is not predicted by the theory. This phenomenon should mean that the β-carotene → Chl-a energy transfer is complex: a "fast" transfer (to some extent uphill-type) is superimposed upon the Förster slow type transfer, involving the participation of the second excited state of Chl-a.

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References

[1] *Duysens, L. N. M.:* Thesis, Utrecht (1952).
[2] *Goedheer, J. C.:* Biochim. Biophys. Acta **172**, 252 (1969).
[3] *Losev, A. P., G. P. Gurinovich:* Biofizika **14**, 110 (1969).
[4] *Teale, F. W. J.:* Nature, **181**, 415 (1958).
[5] *Litvin, F. F., B. A. Gulyayev:* Dokl. A. N. SSSR., **162**, 1184 (1965).
[6] *Sineshchekov, V. A., F. F. Litvin, M. Das:* Photochem. Photobiol. **15**, 187 (1972).
[7] *Singhal, G. S., J. Hevesi, E. Rabinowitch:* J. Chem. Phys. **49**, 5206 (1968).
[8] *Rabinowitch, E., Govindjee:* Photosynthesis, J. Wiley and Sons, New York—London—Sydney—Toronto (1969).
[9] *Förster, Th.:* Fluoreszenz organischer Verbindungen. Vandenhoeck und Ruprecht, Göttingen (1951).
[10] *Szabad, J., L. Szalay:* Fourth Int. Biophys. Congress, Moscow (1972).
[11] *Weber, G.:* Biochem. J. **76**, 335 (1960).
[12] *Bauer, R. K., E. Rabinowitch, L. Szalay, E. Tombácz:* Biophys. J., in press.
[13] *Jacobs, E. E., A. E. Vatter, A. S. Holt:* Arch. Biochem. Biophys. **53**, 228 (1954).
[14] *Zechmeister, L.:* Isomeric carotenoid pigments, in: Fortschritte d. Chem. org. Natursf. **18**, 45 (1961).
[15] *Cherry, R. J., D. Chapman, J. Langelaar:* Trans. Farad. Soc. **44**, 2304 (1968).

ПЕРЕДАЧА ЭНЕРГИИ ЭЛЕКТРОННОГО ВОЗБУЖДЕНИЯ В РАСТВОРЕ ОТ β-КАРОТИНОИДА К ХЛОРОФИЛЛУ

Я. Сабад

Из спектров флуоресценции хлорофилла сенсибилизированных β-каротиноидом определяли R_0, критическое расстояние передачи энергии. В полосе поглощения Соре, в области 400—440 нм это значение зависит от длины волн возбуждающего света и величина R_0 18—22 Å В области 450—500 нм (где в основном поглощает β-каротиноид) $R_0 = 25$ Å. На основе спектров поглощения и флуоресценции $R_0 = 8{,}5$ Å. Предполагается, что в переносе энергии принимает участие через второй синглетный уровень возбуждения хлорофилла одна „быстрая" передача.

* An alternative explanation of this difference may be the incorrect choice of the upper frequency limit of the overlap integral (shown by the dotted line in Fig. 1) in Eq. (1). A higher limit would result in a higher R_0 value.